

Amendments to the Claims:

Claim 1 (currently amended). A method of determining the tertiary structure of a ~~macromolecule~~ protein, comprising the steps of:

imposing physical distance constraints between residues of the ~~macromolecule~~ protein by cross-linking the protein;

fragmenting the ~~macromolecule~~ protein into smaller molecular fragments;

subjecting the fragments to an identification procedure; and

analyzing identification information obtained from the identification procedure to identify cross-link fragments in the protein;

providing a set of candidate three-dimensional conformations for the primary sequence of the protein; and

applying physical distance constraint information for the identified cross-link fragments to the candidate three-dimensional conformations to select one or more of said structures that best fit the distance constraint information ~~provide three-dimensional structural information on the macromolecule.~~

Claim 2 (original). The method of claim 1, wherein the identification procedure comprises mass measurement of the fragments using mass spectrometric analysis.

Claim 3 (original). The method of claim 2, wherein the identification procedure comprises sequence identification.

Claim 4 (cancelled).

Claim 5 (currently amended). The method of claim 1 [4], further comprising:

conducting homology modeling analysis of the selected one or more three-dimensional structures that best fit the distance constraint information ~~hypothetical structures which best fit the distance constraints.~~

Claim 6 (cancelled).

Claim 7 (cancelled).

Claim 8 (currently amended): A method of determining the tertiary structure of a protein, comprising the steps of:

reacting a protein to be analyzed with at least one crosslinking reagent, said reagent comprising at least two reactive groups;

enriching the reaction product for molecules having intramolecular crosslinks;

carrying out proteolysis on the enriched reaction product to yield ~~protein fragments~~ protein fragments;

subjecting the protein fragments to peptide identification analysis; and

analyzing information obtained from the peptide identification analysis to ~~provide information on the three-dimensional structure of the macromolecule~~ identify cross-link fragments in the protein;

providing a set of candidate three-dimensional conformations for the primary sequence of the protein; and

applying physical distance constraint information for the identified cross-link fragments to the candidate three-dimensional conformations to select one or more of said structures that best fit the distance constraint information ~~provide three-dimensional structural information on the macromolecule.~~

Claim 9 (original). The method of claim 8, wherein the crosslinking reagent is a bifunctional crosslinker.

Claim 10 (original). The method of claim 9, wherein the crosslinking reagent is an amine-specific homobifunctional crosslinker.

Claim 11 (original). The method of claim 8, wherein the protein is reacted with a plurality of crosslinking agents having different specificities for reactive sites on the protein.

Claim 12 (currently amended). The method of claim 8, wherein the protein is reacted with a plurality of crosslinking ~~reagents~~ reagents having varying lengths between reactive groups.

Claim 13 (original). The method of claim 1, wherein the reaction with the crosslinker is optimized to produce an average number of one crosslinker modification per macromolecule.

Claim 14 (original). The method of claim 8, wherein the reaction product is enriched for molecules having intramolecular crosslinks by physical removal of proteins having intermolecular crosslinks.

Claims 15-20 (cancelled).

Claim 21 (currently amended). The method of claim 8 20, further comprising:
conducting homology modeling analysis of the selected one or more the three-dimensional structures that best fit the distance constraint information ~~hypothetical structures which fit the distance constraints.~~

Claim 22 (currently amended). The method of claim 1 or 8 20, wherein ~~assigning values is carried out by~~ analyzing information obtained from the peptide identification analysis comprises constructing a virtual library of proteolyzed products which library is indexed by a criteria selected from the group consisting of monoisotopic data and average mass data.

Claim 23 (currently amended). The method of claim 1 or 8 20, wherein ~~hypothetical structures are generated using~~ providing a set of candidate three-dimensional conformations for the full primary sequence of the protein employs a threading program for fold prediction.

Claim 24 (currently amended). The method of claim 1 or 8 20, wherein ~~the hypothetical structures are generated~~ applying physical distance constraint information for the identified cross-link fragments is performed with the use of an equation

$$E_t = \sum_{j=0}^{j \leq i} 0 \text{ if } d_j \leq d_o, \quad d_j - d_o \text{ if } d_j > 0$$

wherein E_t is the total constraint error, d_o is the pairwise distance separation, d_i is the pairwise distance defined by the structure by constraint j and i is the total number of distance constraints.

Claims 25-74 (cancelled)

Claim 75 (new). The method of claim 1 or 8, further comprising performing an initial selection of the candidate three-dimensional conformations by assessing said conformations' compatibility with computed physical properties for the conformations.

Claim 76 (new). The method of claim 75, wherein assessing said conformations' compatibility with computed physical properties for the conformations comprises using at least one technique selected from among: calculating the distribution of hydrophobic/hydrophilic amino acids; mapping a hydrogen-bond network; locating disulfide bridges; functional mapping of mutagenesis data; assessing the complementarity of the hypothetical structure's secondary structure and the secondary structures predicted for the sequence; insuring that critical electrostatic interactions are preserved; identifying sites of van der Waals clashes; and evaluating the sequence-structure-sequence similarity.

Claim 77 (new). The method of claim 1 or 8, wherein the three-dimensional structural information comprises a three-dimensional structure of the macromolecule having a resolution of about 2-5 Angstroms.

Claim 78 (new). A method of determining the tertiary structure of a protein, comprising the steps of:

- (a) cross-linking residues of the protein such that the number of cross-links in the protein is at least about 10% of the number of amino acid residues in the protein;
- (b) fragmenting the protein into molecular fragments;
- (c) subjecting the fragments to a mass spectrometry identification procedure;
- (d) analyzing identification information obtained from the identification procedure to identify distance constraint information about residues in the protein; and
- (e) applying the distance constrain information to candidate three-dimensional conformations to select one or more of said conformations that best fit the distance constraint information.

Claim 79 (new). The method of claim 78, wherein the one or more three dimensional conformations selected in (e) have resolutions of about 2-5 Angstroms.

Claim 80 (new). The method of claim 78, wherein analyzing identification information obtained from the identification analysis comprises constructing a virtual library of proteolyzed products.

Claim 81 (new). The method of claim 78, further comprising, prior to applying the distance constrain information to the candidate three-dimensional conformations, performing an initial selection of the candidate three-dimensional conformations by assessing said conformations' compatibility with computed physical properties for the conformations.

Claim 82 (new). The method of claim 81, wherein assessing said conformations' compatibility with computed physical properties for the conformations comprises using at least one technique selected from among: calculating the distribution of hydrophobic/hydrophilic amino acids; mapping a hydrogen-bond network; locating disulfide bridges; functional mapping of mutagenesis data; assessing the complementarity of the hypothetical structure's secondary structure and the secondary structures predicted for the sequence; insuring that critical electrostatic interactions are preserved; identifying sites of van der Waals clashes; and evaluating the sequence-structure-sequence similarity.